Received: 26 December 2023 | Revised: 5 June 2024 | Accepted: 2 July 2024

DOI: 10.1002/ccr3.9176

CASE REPORT

Clinical Case Reports Open Access WILEY

A family with nine siblings showing signs of Rothmund–Thomson syndrome with two being definitely diagnosed with the syndrome due to homozygous N-terminal mutation of RECQL4

Fatemeh Yadegari[1] | **Aseel Rashid Abed**[2] | **Widad Yadallah Abd Ali**[2] | **Haider Hamza Al-Abedi**[2] | **Shiva Zarinfam**[1] | **Solaleh Aminian**[1] | **Keivan Majidzadeh-A**[1]

[1]Genetics Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran

[2]Warith International Cancer Institute, Karbala, Iraq

Correspondence
Keivan Majidzadeh-A, Genetics Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, No 146, South Gandhi Ave, Vanak Sq., Tehran, Iran.
Email: kmajidzadeh@acecr.ac.ir

Key Clinical Message

This study presents a family with nine children, two of them diagnosed with RTS2 using genetic testing. The other siblings show some of the RTS2 criteria and are suggestive of the syndrome. Such reports help physicians be more alert in dealing with cases of rare syndromes. Timely initiation of genetic counseling and testing once the first child was diagnosed with the syndrome could have prevented the birth of affected siblings by RTS2. Since RTS2 patients could have a severe clinical manifestation as osteosarcoma which probably leads to death at a young age, the importance of genetic testing is even more underlined.

KEYWORDS

biallelic mutations, human, mice, N-terminal homozygous frameshift mutation, *RECQL4* gene, Rothmund–Thomson type II syndrome (RTS2)

1 | INTRODUCTION

Rothmund–Thomson syndrome (RTS) is a rare autosomal recessive disorder with early skin manifestations in life and a significant risk for developing life-threatening cancers, especially osteosarcoma, with the occurrence of one in a million cases. RTS diagnosis should be considered in all patients with osteosarcoma, especially if they showed concurrent skin changes. RTS is characterized by a classic rash in months 3–6, starting as an erythema on cheeks and face, which later spreads to extensor surfaces of extremities. This acute phase is followed by poikiloderma (reticulated hyper and hypopigmentation, telangiectasia, and areas of punctate atrophy) in the chronic phase. If the typical rash is absent, the presence of any two among several criteria, such as sparse hair, eyelashes and/or eyebrows, small stature, gastrointestinal problems like vomiting and diarrhea, dental abnormalities, nail abnormalities, bilateral juvenile cataracts, hyperkeratosis, skeletal abnormalities (including radial, ulnar and patellar defects, as well as osteopenia), and cancers (like osteosarcoma, basal cell carcinoma, and squamous cell carcinoma) are suggestive of RTS. The diagnosis is confirmed when the typical rash is present and/or homozygous pathogenic variants are identified in *ANAPC1*, *DNA2*, *CRIPT*, or *RECQL4*.[1–8] There are two subtypes of RTS, RTS1, and RTS2, each with distinct clinical and genetic characteristics. RTS2 is caused by homozygous or compound heterozygous

mutations in the *RECQL4* gene and is associated with an elevated risk of developing cancer, particularly osteosarcoma.[2,9] *RECQL4* is a member of the human RecQ helicases and plays a crucial role in maintaining the genome's stability during DNA damage repair. The most prominent characteristics differentiating RTSI from RTSII are (I) the absence of osteosarcoma and (II) the development of bilateral juvenile cataracts in all affected individuals.[3] Some RTS1 cases are due to *ANAPC1* mutation, accounting for 10% of RTS patients, while *RECQL4* mutations constitutes 60% of cases and there are also 30% of patients with unknown mutations. In addition to RTS, two other genetic disorders, RAPADILINO syndrome and Baller–Gerold syndrome (BGS), are also caused by biallelic mutations in the *RECQL4* gene. While these syndromes, RTS, RAPADILINO, and BGS, share common symptoms like short stature and radial ray abnormalities, each syndrome also presents specific clinical features.[10] For example, cataracts have only been observed in RTSI, craniosynostosis is a characteristic sign of BGS, and patellar aplasia/hypoplasia and dislocated joints have been observed in RAPADILINO. The clinical differences between these syndromes are depicted in Figure 1.

Apart from *RECQL4*, other members of DNA helicases, such as *BLM* and *WRN*, are linked to genetic syndromes. Mutations in the *BLM* gene lead to Bloom syndrome, while mutations in the *WRN* gene cause Werner syndrome. These syndromes share some similarities in their clinical manifestations with syndromes associated with *RECQL4*.[11] The detailed differences between these syndromes are summarized in Table 1. This study presents a case series of a family from Iraq comprising nine siblings, two being affected with RTS2 and others showing some of the signs of the syndrome.

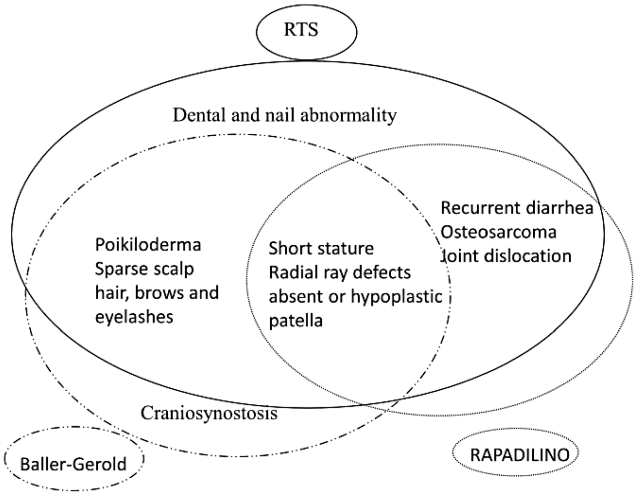

FIGURE 1 The detailed differences between RTS2, RAPADILINO and BGS syndromes.

2 | METHOD

A family with nine children born from a consanguineous marriage sought genetic counseling at the Alwarith Cancer Institute. Unfortunately, three children had passed away due to osteosarcoma, while the remaining children who survived were dealing with deafness, osteosarcoma, and/or poikiloderma (Figure 2). The ages of the living children range from 3 to 13 years. To investigate the genetic cause, we performed WES on index case IV-5, who presented with osteosarcoma, short stature, poikiloderma, and sparse hair. Subsequently, we identified a suspected variant in the proband and confirmed the presence of this mutation in other family members by utilizing PCR and Sanger sequencing of the exon of interest. Their zygosity and clinical manifestations are discussed in Table 2. Unfortunately, DNA samples were unavailable for cases IV-1, IV-2, and IV-7; however, it is assumed that they carried this mutation since they also suffered from osteosarcoma before passing.

3 | RESULTS

3.1 | RTS patients

The Iraqi family consists of nine siblings, with the most common phenotypes observed within this family being osteosarcoma, poikiloderma and/or deafness, and muteness. The parents are healthy heterozygous carriers sharing the same *RecQL4* pathogenic variant as confirmed by the targeted Sanger sequencing. Four children of this family were diagnosed with osteosarcoma; unfortunately, three had passed away at ages 7, 6, and 16. The other child with osteosarcoma is currently 5 years old and showed, deafness and muteness, and poikiloderma. Among the three deceased siblings, two had osteosarcoma as the only abnormality associated with RTS. While the other also exhibited poikiloderma in the early years of life. Of the nine siblings, four were deaf–mute, two had partial hearing loss, and the remaining three had normal hearing (Figure 2). Table 2 provides a summary of the clinical characteristics of all nine siblings.

3.2 | *RECQL4* gene mutations in RTS patients

The 5-year-old son of this family (IV-5) was recently diagnosed with osteosarcoma and presented with other symptoms associated with RTS, including poikiloderma, growth retardation, hearing loss, and sparse hair. The 5-year-old boy was selected for WES to

TABLE 1 The detailed differences between RTS2, Bloom syndrome, and Werner syndrome.

	Bloom's syndrome	Rothmund–Thomson syndrome type2	Werner syndrome
Age at presentation	At birth	3–6 months	Puberty
Growth retardation	<3rd percentile	<5th percentile (66%)	Absence of growth spurt during adolescence
Hair	Normal	Early graying and hair loss	Alopecia, premature hair graying
Skin	Sun-sensitive telangiectatic erythema into a "butterfly distribution" on the face, Café au lait spots	Erythema begins on the face and then spreads to extremities, pigmentation, telangiectasia, poikiloderma	Scleroderma pigmentation alterations, ulceration, trophic ulcerations of the legs
Teeth	Missing lateral incisors	Dental caries, Microdontia, conical teeth (40%)	Normal
Facial dysmorphism	Long and narrow face/prominent ears		+ Pinched facies
Bone abnormality	–	Radial ray defects, hypoplasia of thumbs or patella, osteopenia	Osteoporosis, osteosclerosis, flat feet
Normal intelligence	Mild mental retardation in some patients	Mostly normal	+
Cataract	–	–	Bilateral cataracts
Gonads	Infertility/subfertility	Subfertility	Subfertility
Diabetes	17%	Not increased	71%
Cardiovascular	–	–	Normal anatomy: premature atherosclerosis, myocardial infarction and stroke
Immune system	Decreased immunity with increased susceptibility to infections (otitis)	Normal	Normal
Cancer	Adult epithelial tumors such as colon, breast, and lung cancer; leukemias , lymphomas; sarcomas, Wilms' tumors	Osteosarcoma (32%), skin cancer (2%)	Soft tissue sarcomas, follicular thyroid carcinoma, meningioma, acral lentiginous malignant melanoma, leukemias and osteosarcoma
Most common cause of death	Cancer	Cancer	Cancer and premature cardiovascular disease
Responsible gene	*BLM*	*RECQL4*	*WRN*
Pathogenic variants	Biallelic	Biallelic	Biallelic

investigate potential disease-causing variants in this family. Sequencing showed that the proband carried a homozygous variant in the *RECQL4* gene. This variant is located in a region having sequence similarity to yeast Sld2 (the Sld2-like domain; residues 1–388), which plays a crucial role in DNA replication initiation and cell growth. Only the presence or absence of the suspected variant was tested in this family. Unfortunately, we could not obtain DNA samples from cases IV-1, IV-2, and IV-7, but we presume they carried this mutation since they both passed away due to osteosarcoma.

4 | DISCUSSION

Mutation detection techniques are highly effective diagnostic tools for genetic disorders characterized by a broad range of phenotypic manifestations, such as syndromes associated with RecQ helicase deficiency. Germline mutations in three different human RecQ helicases, known as *RECQL4*, *BLM*, and *WRN*, have been associated with three distinct syndromes: RTSII, WRN, and BLM, respectively. These and other syndromes were discussed in the introduction (Table 1). Interestingly, these

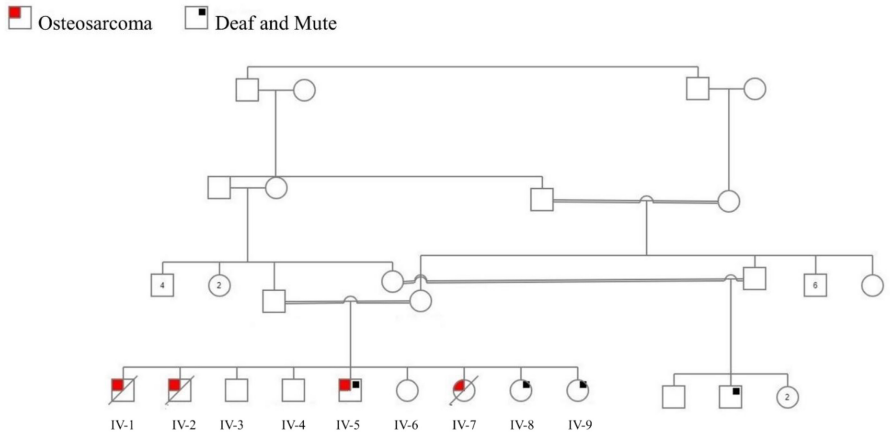

FIGURE 2 Pedigree of the family.

TABLE 2 Age, zygosity and the clinical manifestations of the proband's family.

	Age	Zygosity	Osteosarcoma	Hearing loss	Short stature	Teeth and nail deformity	Poikiloderma	Hair loss
IV-1	Death = 7	Not available	+	–	–	–	–	–
IV-2	Death = 6	Not available	+	–	–	–	–	–
IV-3	3.5	Not tested	–	+	–	–	–	–
IV-4	7.5	Homozygous	–	–	+ (118)	+	+	
IV-5	5	Homozygous	+	+	+ (100)	+	+	+
IV-6	8	Not tested	- (weakness in distal aspect of leg)	–	–	–	–	+
IV-7	Death = 16	Not available	+	–	–	–	+	–
IV-8	13	Heterozygous	–	+	–	–	+	–
IV-9	12	Not tested	–	+	–	–	–	–

three syndromes exhibit overlapping symptoms, such as genomic instability, increased susceptibility to cancer, and premature aging.[11] *RECQL4*, like its counterparts in the RecQ family, harbors a conserved domain positioned within the protein's central region, spanning around 330 amino acids. This domain, known as the helicase domain, encompasses the seven helicase motifs. Unlike other members of this superfamily, *RECQL4* displays distinctive characteristics within its N- and C-terminal domains. The N-terminal part of *RECQL4*, which shares limited homology with yeast Sld2 (the Sld2-like domain; residues 1–388), containing two nuclear localization signals NTS1 and NTS2 (comprising amino acids 37–66 and 363–492),[12,13] a stretch of lysine residues is acetylated by p300,[14] and a mitochondrial localization signal (aa 1–84).[15] Deleting exons 5–8 in mice, encompassing the N-terminus, resulted in embryonic lethality.[16] Conversely, deleting either a single exon 13 or exons 9–13, corresponding to the entire helicase domain, allowed the mice to survive.[17,18] These mouse models provide insights into the rarity of N-terminus mutations of the *RECQL4* gene within the human population because individuals with a defective N-terminus are unlikely to survive beyond the embryonic stage.

Until now, only a few cases of homozygous mutations in the N-terminus of *RECQL4* have been reported. One of these studies explained an 11-month-old Pakistani baby boy with a homozygous frameshift mutation in exon 8 of *RECQL4* (reference sequence NM_004260.3) (c.1453dup, p.Gln485 fs). He showed poikiloderma, sparse hair, gastrointestinal problems, and abnormalities in the nails, teeth, and eyes. Additionally, the child's height and weight measurements were below the 0.4th percentile.[19] Another study had mentioned two Indian children, an 8-year-old male child with a frameshift (c.978_979delTCinsG) and a 4-month-old female child with a splice (c.1132–2A>G) mutation in the *RECQL4* gene. The male child showed recurrent fractures, poikiloderma, and poor growth. The girl child had skeletal defects, poikiloderma, and failure to thrive.[20]

This study presents a homozygous insertion mutation (c.988dup) in exon 5 of *RECQL4*, which was reported pathogenic in Clinvar, leading to a truncated *RECQ4* fragment comprising approximately the first 330 amino acids.

Unlike the complete gene deletion observed in mice, the homozygous c.988dup mutation is compatible with human life but leads to osteosarcoma and death at a young age. intrafamilial variation was observed in this family, which can be due to genetic modifier loci not shared by siblings and/or by different epigenetic cues (Table 2). In this study, four of the nine siblings developed osteosarcoma. It is important to note that although the remaining five children who have not developed cancer are still young, a long-term follow-up is necessary to assess the cancer incidence within this family accurately. At the time of this study, some RTS patients with hearing loss had been reported.[21,22] In this family resulting from a consanguineous marriage, it is noteworthy that four of the nine siblings experienced both deafness and muteness. What's even more fascinating is that among the four siblings who displayed deafness, one (IV-8) had only a single mutation in the *RECQL4* gene (heterozygous mutation). This finding enhances the possibility that since in WES we only find point mutations and small insertions/deletions, other genes rather than *RECQL4* may play a role in the hearing loss presentation in this family.

RTS is an autosomal recessive disease for which there is no treatment, but severe sun protection, cancer surveillance, cataract and orthopedic surgeries if needed, and dental treatments could improve the health status of these patients. These patients have normal intelligence and have a normal lifespan if they develop no cancer. Patients with osteosarcoma have a 5-year survival rate of 60%–70% (as in non-RTS patients).

In conclusion, this study presents a family with nine children, two of them diagnosed with RTS2 using genetic testing. The other siblings show some of the RTS2 criteria and are suggestive of the syndrome. Such reports help physicians be more alert in dealing with cases of rare syndromes. Timely initiation of genetic counseling and testing once the first child was diagnosed with the syndrome could have prevented the birth of affected siblings by RTS2. Since RTS2 patients could have a severe clinical manifestation as osteosarcoma which probably leads to death at a young age, the importance of genetic testing is even more underlined.

AUTHOR CONTRIBUTIONS

Fatemeh Yadegari: Investigation; methodology; software; visualization; writing – original draft; writing – review and editing. **Aseel Rashid Abed:** Conceptualization; investigation; methodology. **Widad Yadallah Abd Ali:** Conceptualization; investigation; methodology; resources. **Haider Hamza Al-Abedi:** Conceptualization; investigation; resources. **Shiva Zarinfam:** Data curation; formal analysis; software; supervision; validation; visualization. **Solaleh Aminian:** Investigation; methodology; validation; visualization; writing – review and editing. **Keivan Majidzadeh-A:** Conceptualization; methodology; project administration; supervision; validation; writing – review and editing.

ACKNOWLEDGMENTS

We thank the patient's family for their participation in this study.

FUNDING INFORMATION

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

CONFLICT OF INTEREST STATEMENT

The author(s) declare no competing interests.

DATA AVAILABILITY STATEMENT

The reference sequence (NM_004260.4) of RECQL4 was obtained from the National Center for Biotechnology Information, NCBI (https://www.ncbi.nlm.nih.gov/). The classification of variants was determined according to the Clinvar database at NCBI (https://www.ncbi.nlm.nih.gov/clinvar/).

ETHICS STATEMENT

This study was approved by ethical committee of the Avicenna Research Institute, ACECR.

IR.ACECR.Avicenna.REC.1396.24.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Keivan Majidzadeh-A https://orcid.org/0000-0002-8811-0997

REFERENCES

1. Bachrati CZ, Hickson ID. RecQ helicases: suppressors of tumorigenesis and premature aging. *Biochem J*. 2003;374(3):577-606.
2. Kitao S, Shimamoto A, Goto M, et al. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat Genet*. 1999;22(1):82-84.
3. Larizza L, Roversi G, Volpi L. Rothmund-Thomson syndrome. *Orphanet J Rare Dis*. 2010;5:1-16.
4. Lindor NM, Furuichi Y, Kitao S, Shimamoto A, Arndt C, Jalal S. Rothmund-Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. *Am J Med Genet*. 2000;90(3):223-228.
5. Martins DJ, Di Lazzaro Filho R, Bertola DR, Hoch NC. Rothmund-Thomson syndrome, a disorder far from solved. *Frontiers in Aging*. 2023;4:1296409.

6. Vennos EM, James WD. Rothmund-Thomson syndrome. *Dermatol Clin*. 1995;13(1):143-150.
7. Wang LL, Levy ML, Lewis RA, et al. Clinical manifestations in a cohort of 41 Rothmund-Thomson syndrome patients. *Am J Med Genet*. 2001;102(1):11-17.
8. Wang, L. L., & Plon, S. E. (2020). *Rothmund-Thomson Syndrome*. GeneReviews, University of Washington.
9. Lu L, Jin W, Wang LL. RECQ DNA helicases and osteosarcoma. *Adv Exp Med Biol*. 2020;1258:37-54.
10. Larizza L, Magnani I, Roversi G. Rothmund–Thomson syndrome and RECQL4 defect: splitting and lumping. *Cancer Lett*. 2006;232(1):107-120.
11. Chu WK, Hickson ID. RecQ helicases: multifunctional genome caretakers. *Nat Rev Cancer*. 2009;9(9):644-654.
12. Burks LM, Yin J, Plon SE. Nuclear import and retention domains in the amino terminus of RECQL4. *Gene*. 2007;391(1–2):26-38.
13. Croteau DL, Singh DK, Ferrarelli LH, Lu H, Bohr VA. RECQL4 in genomic instability and aging. *Trends Genet*. 2012;28(12):624-631.
14. Dietschy T, Shevelev I, Pena-Diaz J, et al. p300-mediated acetylation of the Rothmund-Thomson-syndrome gene product RECQL4 regulates its subcellular localization. *J Cell Sci*. 2009;122(8):1258-1267.
15. De S, Kumari J, Mudgal R, et al. RECQL4 is essential for the transport of p53 to mitochondria in normal human cells in the absence of exogenous stress. *J Cell Sci*. 2012;125(10):2509-2522.
16. Ichikawa K, Noda T, Furuichi Y. Preparation of the gene targeted knockout mice for human premature aging diseases, Werner syndrome, and Rothmund-Thomson syndrome caused by the mutation of DNA helicases. *Nihon Yakurigaku Zasshi Folia Pharmacologica Japonica*. 2002;119(4):219-226.
17. Hoki Y, Araki R, Fujimori A, et al. Growth retardation and skin abnormalities of the Recql4-deficient mouse. *Hum Mol Genet*. 2003;12(18):2293-2299.
18. Mann MB, Hodges CA, Barnes E, Vogel H, Hassold TJ, Luo G. Defective sister-chromatid cohesion, aneuploidy and cancer predisposition in a mouse model of type II Rothmund–Thomson syndrome. *Hum Mol Genet*. 2005;14(6):813-825.
19. Bhoyrul B, Lindsay H, Robinson R, et al. Pili annulati in a case of Rothmund-Thomson syndrome with a novel frameshift mutation in RECQL4. *Eur Acad Dermato Venereo: JEADV*. 2017;32(6):e221-e223.
20. Yadav S, Thakur S, Kohlhase J, Bhari N, Kabra M, Gupta N. Report of two novel mutations in Indian patients with Rothmund–Thomson syndrome. *J Pediatr Genet*. 2019;8(3):163-167.
21. Beghini A, Castorina P, Roversi G, Modiano P, Larizza L. RNA processing defects of the helicase gene RECQL4 in a compound heterozygous Rothmund–Thomson patient. *Am J Med Genet A*. 2003;120 A(3):395-399.
22. Siitonen HA, Sotkasiira J, Biervliet M, et al. The mutation spectrum in RECQL4 diseases. *Eur J Hum Genet*. 2009;17(2):151-158. doi:10.1038/ejhg.2008.154

How to cite this article: Yadegari F, Abed AR, Abd Ali WY, et al. A family with nine siblings showing signs of Rothmund–Thomson syndrome with two being definitely diagnosed with the syndrome due to homozygous N-terminal mutation of RECQL4. *Clin Case Rep*. 2024;12:e9176. doi:10.1002/ccr3.9176